

CLAIMS

1. A method of lysing an acid-fast bacterium to extract a gene from the acid-fast bacterium, comprising:
 - 5 heating the acid-fast bacterium in a liquid containing a non-ionic detergent at a temperature below a boiling point of the liquid.
2. The method according to claim 1, wherein the heating temperature is not less than 70°C and less than 100°C.
- 10 3. The method according to claim 1 or 2, wherein the heating is performed for 1 to 30 minutes.
4. The method according to claim 1, wherein the heating is performed at 96°C for 10 minutes.
- 15 5. The method according to any one of claims 1 to 4, wherein a pH of the liquid is in a range from 7.0 to 12.0.
- 20 6. The method according to any one of claims 1 to 5, wherein a concentration of the non-ionic detergent in the liquid is 0.01 to 10 wt%.
7. The method according to any one of claims 1 to 6, wherein the non-ionic detergent is at least one selected from the group consisting of D-sorbitol fatty acid esters, polyoxyethyleneglycol sorbitan alkyl esters, and 25 polyoxyethyleneglycol p-t-octylphenyl ethers.
8. The method according to any one of claims 1 to 7, wherein the liquid further contains a metal chelating agent.
- 30 9. The method according to claim 8, wherein a concentration of the metal chelating agent in the liquid is 0.1 to 100 mM.
10. The method according to claim 8 or 9, wherein the metal chelating agent 35 is at least one selected from the group consisting of ethylenediaminetetraacetic acid (EDTA), ethylene glycol bis(β-aminoethyl ether) - N,N,N',N'-tetraacetic acid (EGTA), diaminocyclohexane tetraacetic

acid, o-phenanthroline, and salicylic acid.

11. The method according to any one of claims 1 to 10, wherein the acid-fast bacterium to be lysed is at least one selected from the group consisting of *M. avium*, *M. intracellulareae*, *M. gordonae*, *M. tuberculosis*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, *M. bovis*, *M. scrofulaceum*, *M. paratuberculosis*, *M. phlei*, *M. marinum*, *M. simiae*, *M. scrofulaceum*, *M. szulgai*, *M. leprae*, *M. xenopi*, *M. ulcerans*, *M. lepraemurium*, *M. flavescens*, *M. terrae*, *M. nonchromogenicum*, *M. malmoense*, *M. asiaticum*, *M. vaccae*, *M. gastri*, *M. triviale*, *M. haemophilum*, *M. africanum*, *M. thermoresistable*, and *M. smegmatis*.

12. The method according to any one of claims 1 to 11, wherein a biological sample containing the acid-fast bacterium is at least one selected from the group consisting of sputum, spinal fluid, feces, saliva, blood, tissues, and urine.

13. A method of amplifying or detecting a gene of an acid-fast bacterium specifically, comprising:
lysing an acid-fast bacterium by the method according to any one of claims 1 to 12 to extract a gene of the acid-fast bacterium; and
amplifying or detecting the gene specifically using the extracted gene as a sample.

14. A method of lysing an acid-fast bacterium to extract a gene from the acid-fast bacterium, comprising:
causing lipolysis by treating the acid-fast bacterium with lipase, and
heating the acid-fast bacterium in the presence of a non-ionic detergent.

15. The method according to claim 14, wherein the heating also serves to deactivate the lipase.

16. The method according to claim 14 or 15, wherein the lipolysis and the heating are performed in a buffer.

17. The method according to any one of claims 14 to 16, wherein the

lipolysis and the heating are performed in a same container as a closed system.

18. The method according to any one of claims 14 to 17, wherein the heating
5 is performed after the lipolysis.

19. The method according to claim 18, wherein the lipolysis is caused at a
pH of 4 to 8 and at a temperature of 37°C to 60°C for 5 to 30 minutes, and the
heating is performed at a temperature of 37°C to 100°C for 5 to 30 minutes.
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20. The method according to any one of claims 14 to 19, wherein the
lipolysis and the heating are performed simultaneously.

21. The method according to claim 20, wherein the lipolysis and the heating
15 are performed at a pH of 4 to 8 and at a temperature of 37°C to 60°C for 5 to
30 minutes.

22. The method according to claim any one of claims 16 to 21, wherein a
concentration of the lipase in the buffer is 10 to 10000 units/ml.
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23. The method according to any one of claims 14 to 22, wherein the
non-ionic detergent is at least one selected from the group consisting of
D-sorbitol fatty acid esters, polyoxyethyleneglycol sorbitan alkyl esters, and
polyoxyethyleneglycol p-t-octylphenyl ethers.
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24. The method according to any one of claims 16 to 23, wherein a
concentration of the non-ionic detergent in the buffer is 0.01 to 10 wt%.

25. The method according to any one of claims 14 to 24, wherein the heating
30 is performed in the presence of a metal chelating agent in addition to the
non-ionic detergent.

26. The method according to claim 25, wherein the metal chelating agent is
at least one selected from the group consisting of ethylenediaminetetraacetic
35 acid (EDTA), glycol ether diaminetetraacetic acid (EGTA), and
1,2-cyclohexanediaminetetraacetic acid (CyDTA).

27. The method according to claim 25 or 26, wherein a concentration of the metal chelating agent in the buffer is 0.1 to 2.0 mM.
28. The method according to any one of claims 14 to 27, wherein the
5 acid-fast bacterium to be lysed is at least one selected from the group consisting of *M. avium*, *M. intracellularea*, *M. gordonae*, *M. tuberculosis*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, *M. bovis*, *M. scrofulaceum*, *M. paratuberculosis*, *M. phlei*, *M. marinum*, *M. simiae*, *M. scrofulaceum*, *M. szulgai*, *M. leprae*, *M. xenopi*, *M. ulcerans*, *M. lepraemurium*, *M. flavescens*,
10 *M. terrae*, *M. nonchromogenicum*, *M. malmoense*, *M. asiaticum*, *M. vaccae*, *M. gastri*, *M. triviale*, *M. haemophilum*, *M. africanum*, *M. thermoresistable*, and *M. smegmatis*.
29. The method according to any one of claims 14 to 28, wherein a biological
15 sample containing the acid-fast bacterium is at least one selected from the group consisting of sputum, spinal fluid, feces, saliva, blood, tissues, swab, liquid obtained by gastrolavage, and urine.
30. A method of amplifying or detecting specifically a gene of an acid-fast
20 bacterium, comprising:
 lysing an acid-fast bacterium by the method according to any one of claims 14 to 29 to extract a gene of the acid-fast bacterium; and
 amplifying or detecting the gene specifically using the extracted gene as a sample.